

## Ethanollic *Sisyrinchium palmifolium* L. Extract as Natural Preservative for Indonesian Tofu Preparation

### Ekstrak Etanol *Sisyrinchium palmifolium* L. sebagai Pengawet Alami untuk Olahan Tahu Indonesia

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#### ABSTRACT

Tofu is a food product that is easily contaminated by microbial due to its water content. Some bacteria that usually grow in tofu are *Escherichia coli*, *Bacillus cereus*, or *Staphylococcus aureus*. Preservatives are added to solve the common storage problem. However, some manufacturers use hazardous substances, such as formalin or other chemical substances, as a preservative. Tiwai onion (*Sisyrinchium palmifolium* L.) is a plant that grows in Borneo and has a broad range of antibacterial activity. This study aimed to examine the activity and effectiveness of *Sisyrinchium palmifolium* extract as a preservative in tofu. *Sisyrinchium palmifolium* was extracted using the maceration method with ethanol three times. The concentrated ethanol extract has a 5% water content and was used for the next step. According to the Clinical and Laboratory Standard Institute (CLSI method), the test was done with agar diffusion and broth microdilution methods. The test solution was diluted using DMSO 5% as the solvent, and tetracycline HCl solution as a positive control was diluted using NaCl 0.9%. Agar diffusion method was done with *Sisyrinchium palmifolium* ethanol extract 10000 µg/mL and tetracycline HCl 50 µg/mL. The microdilution method was done with *Sisyrinchium palmifolium* ethanol extract with an initial concentration of 40000 µg/mL and tetracycline HCl 2000 µg/mL. The results showed that *Sisyrinchium palmifolium* ethanolic extract has antibacterial activity with the minimum inhibitory concentration value of 5000 µg / mL. Then, the effectivity of concentrated ethanol extract of *Sisyrinchium palmifolium* as a preservative in tofu was tested by determining Total Plate Count at an incubation temperature of 37, 25, and 4 °C in comparison to potassium

sorbate as control. Furthermore, organoleptic evaluation was observed at 25 and 4 °C. The results showed that *Sisyrinchium palmifolium* ethanolic extract was effective as an alternative preservative for tofu at a concentration of 5000 µg/mL. In conclusion, ethanolic extract of *Sisyrinchium palmifolium* could serve as a novel candidate and effective preservative in tofu.

**Keywords:** Antimicrobial, *Sisyrinchium palmifolium* (Mill.) Urb., extract, preservative, tofu

### ABSTRAK

Tahu merupakan produk pangan yang mudah tercemar mikroba karena kandungan airnya yang tinggi. Beberapa bakteri yang biasanya tumbuh pada tahu adalah *Escherichia coli*, *Bacillus cereus*, atau *Staphylococcus aureus*. Pengawet ditambahkan untuk mengatasi masalah penyimpanan yang umum. Namun, beberapa produsen menggunakan bahan berbahaya seperti formalin atau bahan kimia lain sebagai pengawet. Bawang tiwai (*Sisyrinchium palmifolium* L.) merupakan tanaman yang tumbuh di Kalimantan dan telah dilaporkan memiliki aktivitas antibakteri yang luas. Tujuan penelitian ini adalah untuk mengetahui aktivitas dan efektivitas ekstrak bawang tiwai sebagai pengawet tahu. Ekstraksi bawang tiwai menggunakan metode maserasi dengan pelarut etanol sebanyak 3 kali. Ekstrak etanol pekat memiliki kadar air 5% dan digunakan untuk tahap selanjutnya. Menurut Clinical and Laboratory Standard Institute (metode CLSI), pengujian dilakukan dengan metode difusi agar dan metode mikrodilusi kaldu. Larutan uji diencerkan menggunakan DMSO 5% sebagai pelarut dan larutan tetrasiklin HCl sebagai kontrol positif diencerkan menggunakan NaCl 0,9%. Metode difusi agar dilakukan dengan ekstrak etanol bawang tiwai 10000 µg/mL dan tetrasiklin HCl 50 µg/mL. Metode mikrodilusi dilakukan dengan ekstrak etanol bawang tiwai dengan konsentrasi awal 40000 µg/mL dan tetrasiklin HCl 2000 µg/mL. Hasil penelitian menunjukkan bahwa ekstrak etanol bawang tiwai memiliki aktivitas antibakteri dengan nilai konsentrasi hambat minimal 5000 µg/mL. Kemudian, efektivitas ekstrak etanol pekat bawang tiwai sebagai pengawet tahu diuji dengan menentukan *Total Plate Count* pada suhu inkubasi 37, 25, dan 4° C dibandingkan dengan kalium sorbat sebagai kontrol. Kemudian dilakukan evaluasi organoleptic pada suhu 4 dan 25 °C. Hasil penelitian menunjukkan bahwa ekstrak etanol bawang tiwai efektif sebagai pengawet alternatif tahu pada konsentrasi 5000 µg/mL. Kesimpulannya, ekstrak etanol bawang tiwai dapat menjadi kandidat pengawet yang baru dan efektif pada tahu.

**Kata kunci:** Antimikroba, ekstrak, *Sisyrinchium palmifolium* L., pengawet, tahu.

### Introduction

Tofu is one of food product which is easily spoilage and contaminated and susceptible to cause foodborne disease due to its high-water

content (Rekha dan Vijayalaksmic, 2013). A variety of microorganisms may cause this spoilage for example *Escherichia coli*, *Staphylococcus aureus*, as well as *Bacillus cereus* (Kadariya et al., 2014;

Rossi *et al.*, 2016; Quinland, 2013). The use of preservative to enhance tofu shelf-life is great interest for food industry, in which mostly chemical-preservative material. A solution of formaldehyde (Formalin; CH<sub>2</sub>O) was widely used for tofu handling and processing.

Though its effectiveness as preservative, it has been showed that exposure to formaldehyde may cause adverse human health effect such as skin irritation, allergy, pneumonia, and cancer (Norliana *et al.*, 2009). Another preservation method widely used for tofu handling and processing are immersion of tofu in solution containing salt such as potassium sorbate or sodium chloride, or immersion of tofu in traditional mixture such as turmeric and lime solution or lime and kitchen salt solution (Jubayer and Faruque, 2013).

Tiwai onion (*Sisyrinchium palmifolium* L.) is a herbal plant whose traditionally used as natural product to prevent and cure hypertension, diabetic, and acnes (Febrinda *et al.*, 2014). It has been reported the extract of *Sisyrinchium palmifolium* showed inhibitory activity against *Staphylococcus aureus* (Ifesan and Voravuthikunchai 2009; Ifesan *et al.*, 2009) and other bacteria such as *Shigella boydii* (Panda *et al.*, 2016). There was no reported research up to now regarding the use of the *Sisyrinchium palmifolium* as preservative and additive in tofu processing and preparation. We carried out this study to examine the ability of

*Sisyrinchium palmifolium* extract as preservative in tofu.

## Research Method

### Chemical and Reagents

*Sisyrinchium palmifolium* bulb was obtained freshly from Bandung, Indonesia, cleaned and characterized and dried prior to use. Tofu was freshly obtained from Yun Yi Industry, *Escherichia coli* (ATCC 8739), *Bacillus cereus* (ATCC 1178) were obtained from American Type Culture Collection. Nutrient Agar (NA), Nutrient Broth (NB), Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), tryptone, yeast extract was used as medium for bacterial culture, tetracycline as standard material, sodium hydroxide, potassium sorbate, DMSO and all commercially available reagents were analytical grade and used as purchased (Merck, Sigma-Aldrich).

### Characterization of *Sisyrinchium palmifolium* Bulb

The collected dried *Sisyrinchium palmifolium* bulb was done through general phytochemical screening test (alkaloid, flavonoid, saponin, tannin, quinone, steroid/triterpenoid) according to protocols in Indonesian Herbal Pharmacopoeia (MOH, 2008).

### Preparation of Ethanolic *Sisyrinchium palmifolium* Extract

The powdered and dried *Sisyrinchium palmifolium* bulb was extracted using maceration method using three times of ethanol (1:5). The

extract was filtered using filter paper and the filtrates were evaporated under reduced pressure in a rotary evaporator. The extract was dissolved in 5% DMSO (in 0.9% NaCl) before use. The end concentration of extract is 40000 µg/mL and 10000 µg/mL.

#### *Preparation of Suspended Microbe*

The preparation of suspended microbe was adapted from CLSI (CLSI, 2012). There are two microbes used in this study, *Eschericia coli* and *Bacilus cereus*. The microbes grown on the surface of agar plate was slowly taken and suspended in nutrient agar media and incubated at 37°C. The suspended microbe was made by selected the microbe of interest and suspended in nutrient broth media (1.8% in water). Dilution was made using NaCl solution (0.9% in water) until it reaches the 0.5 McFarland value or the UV-Vis spectrophotometer absorbance range between 0.08-0.13 at 625 nm. The total colony forming unit was measured before the antimicrobial activity determination.

#### *Antimicrobial Activity of Ethanolic Extract Determination*

##### 1. Agar diffusion method

Antimicrobial activity was evaluated using paper disc agar diffusion. Ethanolic *Sisyrrinchium palmifolium* extract 40000 µg/mL was diluted in 5% DMSO until the final concentration of the test solution are 50, 100 and 200 µg/ml, respectively. Tetracycline HCl (50 µg/ml in 0.9%

NaCl) was used as a negative control meanwhile 0.9% NaCl was used as positive control. Ten microliters of the test solution (extracts, tetracycline HCl and 0.9% NaCl) were then added to sterile filter paper disc and placed on the dishes containing microbe of interest. The dishes were incubated for 18-24 hours at 37°C. The means of the diameter of the inhibition zone were calculated.

##### 2. Microdilution method

The minimal inhibitory concentration (MIC) of ethanolic extract was determined using microdilution (96 round-bottomed-microwell plate) method according to Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2012; CLSI, 2016).

Ethanolic *Sisyrrinchium palmifolium* extract was diluted in 5% DMSO. Ten microliters of each bacterial strains (diluted 1:20 with nutrient broth) was added to 100 µL MHB media, supplemented with ethanolic extract (2-fold dilution) at concentration from 20000, 10000, 5000, 2500, 1250, 625, 312, 156, 78, 39 µg/mL. Control with tetracycline HCl 2000 µg/ml and without the extracts were carried out under similar condition. The plate was then incubated for 16-20 hours at 37°C. Minimal inhibitory concentrations were observed as the lowest concentration of the extracts that produce a complete inhibition of colony growth. The experiment was

conducted in three independent measurements.

#### Determination of Antimicrobial Activity of Ethanolic Extract in Tofu

Total plate count was used to determine the antimicrobial activity of the extract. Potassium sorbate (1% in sterile water solution) was used as control. The tofu was immersed in water, after several hours, the water was carefully taken and used as samples for determining the antimicrobial activity. Ethanolic *Sisyrinchium palmifolium* extract which produce activity on the previous assay was used as test substance. Plate count agar (PCA) was used for total plate count. Test substance and the control were added in comparison of 15:1:1. All the plates were incubated at different condition to mimic the storage of tofu, 18-24 hours (37°C), 72 hours (25°C), 168 hours or 7 days (4°C). Organoleptic evaluation was carried out every single day of the assay for storage in 25°C and 4°C.

#### Results and Discussion

*Sisyrinchium palmifolium* bulb is herbal plant that may be produced and cultivated under different condition. Characterization and phytochemical screening were applied to convince standardize bulb was used in this study. Characterization and phytochemical screening of dried *Sisyrinchium palmifolium* bulb is given in Table 1 and Table 2. The phytochemical screening showed *Sisyrinchium palmifolium* bulb

contains flavonoid, polyphenol, saponin, quinone and steroid/triterpenoid.

Ifesan and collogue reported antimicrobial activity of *Eleutherine americana* on *Staphylococcus aureus* from food (Ifesan and Voravuthikunchai 2009; Ifesan *et al*, 2009). Our study confirms that ethanolic *Sisyrinchium palmifolium* extract had antibacterial activity towards *Escherichia coli* and *Bacillus cereus*. It was found that at the concentration of 10000 µg/mL was optimum concentration for the inhibition for both E. coli and B. cereus (Table 3).

**Table 1.** Phytochemical screening results of dried *Sisyrinchium palmifolium* bulb

Phytochemical group	Result
Alkaloid	-
Flavonoid	+
Polyphenol	+
Saponin	+
Quinone	+
Tannin	-
Steroid/ Triterpenoid	+

+ = Presence of the phytochemical group, - = absence of phytochemical group

**Table 2.** Characterization results of dried *Sisyrinchium palmifolium* bulb

Parameter (unit)	Result (%)
Water content	5.0
Total ash	0.768
Acid insoluble ash	0.675
Water extractable matter	4.924
Ethanol extractable matter	5.591

Encouraged by these results, our next effort was to determine the MIC value of the ethanolic *Sisyrinchium palmifolium* extract on both *Escherichia*

*coli* and *Bacillus cereus*. Broth microdilution method was selected in determining the MIC value. The results of microdilution methods were shown in Table 4 for *Escherichia coli* and Table 5 for *Bacillus cereus*. It was showed that ethanolic *Sisyrrinchium palmifolium* extract has MIC value of 5000 µg/mL for *Escherichia coli* and 10000 µg/mL for *Bacillus cereus*. We then conclude to use 5000 µg/mL as the minimum concentration for antimicrobial activity evaluation on tofu products.

Total plate count (TPC) was used to evaluate the effectiveness of the extract as preservative because the reliability and the reproducibility of the method (Table 6-8). Result showed that

the addition of extract decreases the number of total colonies. Three temperatures used in the experiment to count the TPC of tofu (37, 25, and 4°C). The results showed that *Sisyrrinchium palmifolium* extract was able to suppress the growth of microbial in tofu either in reduced temperature as well as in room temperature. Tofu stored in reduced temperature showed lower total colony count compared to tofu stored in room temperature during observation time. However, there is a limitation storage time for treated tofu in refrigerator. The *Sisyrrinchium palmifolium* extract showed good activity on tofu compared to potassium sorbate.

**Table 3.** Inhibition diameter for each tested bacterium. The absence of bacteria is denotated with minus (-). (n=2)

Sample	Inhibition diameter (mm) against	
	<i>Escherichia coli</i>	<i>Bacillus cereus</i>
<i>Sisyrrinchium palmifolium</i> extract (10000 µg/mL)	10±2	12.5±1.5
	12±1	13.5±2
Tetracycline HCl (50 µg/mL)	14±3	18±1
	15±1.5	21,5±2.5
0.9% NaCl	-	-
	-	-

**Table 4.** Broth microdilution result of *Sisyrrinchium palmifolium* extract on *Escherichia coli*

	(-)	(+)	1	2	3	4	5	6	7	8	9	10
Extract	+	-	-	-	-	+	+	+	+	+	+	+
	+	-	-	-	-	+	+	+	+	+	+	+
	+	-	-	-	-	+	+	+	+	+	+	+

Note: (-) = negative control, (+) = positive control 50 µg/mL, - = no bacteria suspension observed, + = bacteria suspension observed, i.e., 1 = 20000 µg/mL, 2 = 10000 µg/mL, 3 = 5000 µg/mL, 4 = 2500 µg/mL, 5 = 1250 µg/mL, 6 = 625 µg/mL, 7 = 312 µg/mL, 8 = 156 µg/mL, 9 = 78 µg/mL, 10 = 39 µg/mL

**Table 5.** Broth microdilution result of *Sisyrrinchium palmifolium* extract on *Bacillus cereus*

	(-)	(+)	1	2	3	4	5	6	7	8	9	10
Extract	+	-	-	-	+	+	+	+	+	+	+	+
	+	-	-	-	+	+	+	+	+	+	+	+
	+	-	-	-	+	+	+	+	+	+	+	+

Note: (-) = negative control, (+) = positive control µg/mL, - = no bacteria suspension observed, + = bacteria suspension observed, i.e., 1 = 20000 µg/mL, 2 = 10000 µg/mL, 3 = 5000 µg/mL, 4 = 2500 µg/mL, 5 = 1250 µg/mL, 6 = 625 µg/mL, 7 = 312 µg/mL, 8 = 156 µg/mL, 9 = 78 µg/mL, 10 = 39 µg/mL

**Table 6.** TPC results of sample for 18-24 hours at 37°C

Sample	Total colony count (10 <sup>5</sup> CFU/mL) at	
	18 hours	24 hours
Tofu	>200	>200
Tofu + Potassium sorbate 1%	41	42
Tofu + <i>Sisyrrinchium palmifolium</i> extract	42	45

**Table 7.** TPC results of sample in 3 days at 25°C

Sample	Total colony count (10 <sup>5</sup> CFU/mL) at		
	24 hours	48 hours	72 hours
Tofu	2	5	15
Tofu + Potassium sorbate 1%	1	3	4
Tofu + <i>Sisyrrinchium palmifolium</i> extract	0	2	2

**Table 8.** TPC results of sample in 7 days at 4°C

Sample	Total colony count (10 <sup>5</sup> CFU/mL) at				
	24 hours	48 hours	72 hours	96 hours	168 hours
Tofu	0	4	7	10	11
Tofu + Potassium sorbate 1%	0	3	3	3	3
Tofu + <i>Sisyrrinchium palmifolium</i> extract	0	0	0	0	1

Total plate count (TPC) was used to evaluate the effectiveness of the extract as preservative because the reliability and the reproducibility of the method (Table 6-8). Result showed that the addition of extract decreases the number of total colonies. Three temperatures used in the experiment to count the TPC of tofu (37, 25 and 4 °C). The results showed that *Sisyrrinchium palmifolium* extract was able to suppress

the growth of microbial in tofu either in reduced temperature as well as in room temperature. Tofu stored in reduced temperature showed lower total colony count compared to tofu stored in room temperature during observation time. However, there is a limitation storage time for treated tofu in refrigerator. The *Sisyrrinchium palmifolium* extract showed good activity on tofu compared to potassium sorbate.

**Table 9.** Organoleptic evaluation on tofu products at 25°C

Sample	Organoleptic evaluation at definite time		
	24 hours	48 hours	72 hours
Tofu	Tainted in odor and texture	Tainted in odor and texture	Tainted in odor and texture, black spot
Tofu + Potassium sorbate 1%	Untainted in odor and texture	Untainted in odor and bit texture	Untainted in odor and bit in texture
Tofu + <i>Sisyrrinchium palmifolium</i> extract	Bit of rancid odor and untainted in texture	Bit of rancid odor and tainted in texture	Bit of rancid odor and tainted in texture

**Table 10.** Organoleptic evaluation on tofu products at 4°C

Sampel	Organoleptic evaluation at definite time				
	24 hours	48 hours	72 hours	96 hours	168 hours
Tofu				Rancid odor, untainted in texture	Tainted in odor and texture
Tofu + Potassium sorbate	Untainted in odor and texture	Untainted in odor and texture	Untainted in odor and texture	Rancid odor, untainted in texture	Rancid odor, untainted in texture
Tofu + <i>Sisyrrinchium palmifolium</i> extract				Untainted in odor and texture	Rancid odor, untainted in texture

To obtain deeper insight into the acceptability of tofu as food products, the organoleptic evaluation was conducted for storage in 25 and 4 °C (Table 9-10). It showed that the *Sisyrrinchium palmifolium* extract capable to maintain the texture and odor of tofu during the storage and therefore may increasing the self-life of tofu.

### Conclusion

In summary, *Sisyrrinchium palmifolium* extract was found to be a good candidate as substitution of chemical preservative in tofu processing and preparation.

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